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To cite this article: Tiziano Bo, Massimo Cammarata, Alberto Doretto & Stefano Fenoglio (2020): How organic pollution and habitat alteration influence the trophic habits of *Perlodes intricatus* (Pictet, 1841) in alpine rivers?, *Aquatic Insects*, DOI: [10.1080/01650424.2019.1708406](https://doi.org/10.1080/01650424.2019.1708406)

To link to this article: <https://doi.org/10.1080/01650424.2019.1708406>



Published online: 09 Jan 2020.



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How organic pollution and habitat alteration influence the trophic habits of *Perlodes intricatus* (Pictet, 1841) in alpine rivers?

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ABSTRACT

In this study, we analysed the diet of two populations of *Perlodes intricatus* (Pictet, 1841) inhabiting river sections characterised by different environmental quality. Macroinvertebrate communities and nymphal gut content data were acquired in a nearby pristine environment and in a station compromised by sewage discharges. No differences in size were detected between these populations. Both populations select positively Chironomidae as prey, but marked differences are evident between their diet. Nymphs from pristine station feed on a wide range of prey, but also include algae and organic detritus. By contrast, nymphs from polluted station show a different and restricted trophic spectrum, likely because of the reduced prey availability. Interestingly, the occurrence of fine organic detritus in these latter nymphs is much higher, evidencing a shift to a more collector-gatherer feeding. This study shows that some Perlodidae can survive and develop in contaminated environments, also due to their trophic plasticity.

ARTICLE HISTORY

Received 28 October 2019
Revised 22 November 2019
Accepted 18 December 2019

KEYWORDS

Plecoptera; Perlodidae; gut content; food items; northwestern Italy

Introduction

The study of feeding habits of stream insects has attracted an increasing attention in recent years because of its importance in the study of food webs, energy transfer and new frontiers of biomonitoring (Gamboa, Chacón, and Segnini 2009; López-Rodríguez, Tierno de Figueroa, Bo, Moggi, and Fenoglio 2012; Merritt, Fenoglio, and Cummins 2017). This topic is particularly important in alpine lotic systems, characterised by harsh environmental conditions and small fish populations, where large-sized insect predators play a key role in the benthic community (Dudgeon 2000; Wipfli and Gregovich 2002). In these environments, the trophic ecology of aquatic invertebrates is remarkably poorly investigated, representing approximately 7% of published studies (Niedrist and Füreder 2017), despite its importance and function. In alpine lotic systems, top predators are mainly represented by Plecoptera Systelognatha, belonging to the Perlidae and Perlodidae families (Tachet, Richoux,

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Bournaud, and Usseglio-Polatera 2010). To underline their importance as predators, Allan (1983) reported that in a Rocky Mountain stream, stoneflies consume a quantity of prey (as dry mass) that is equivalent to half of that consumed by large vertebrate predators (i.e., trout). A recent paper by Tierno de Figueroa and López-Rodríguez (2019) reviewed trophic habits of large-sized Plecoptera, reporting that although these organisms are generally considered predators, some variability may occur among different species and populations. Some studies underlined trophic plasticity in carnivorous stoneflies during ontogenesis, evidencing that larger nymphs consume a broader range of prey items (Fenoglio, Bo, López-Rodríguez, Tierno de Figueroa, and Malacarne 2009; Fenoglio, Bo, López-Rodríguez, and Tierno de Figueroa 2010) or a shift from a more detritivorous to a strictly carnivorous diet (Lucy, Costello, and Giller 1990; Bo et al., 2007), as demonstrated also for other aquatic insects (Céréghino 2002; Blois 2006). Other studies reported that the diet of predaceous stoneflies could be influenced by season, habitat typology and complexity, sympatry with other predators (Malmqvist, Sjöström, and Frick 1991; Thorp, Monroe, Thorp, Wellnitz, and Poff 2007; Bo et al. 2010) but interestingly almost no information is available about the influence of habitat deterioration on stoneflies trophic habits. Plecoptera, and in particular large-sized Systellognatha, are well known as important indicators in biomonitoring because of their sensitivity of environmental quality (Fochetti and Tierno de Figueroa 2008b), so that it is unusual to find a population in a contaminated environment. Since we recently recorded a population of *Perlodes intricatus* (Pictet, 1841) in an organic polluted alpine river, we decided to better investigate its diet under such impacted conditions. *Perlodes intricatus* is a large-sized European orophilous stonefly, distributed in Italy in the Alps and in Northern Apennines from 800 to 2700 m a.s.l., above sea level, (Fochetti and Tierno de Figueroa 2008a) and usually associated with high quality environments (Bona, Falasco, Fenoglio, Iorio, and Badino 2008; Doretto et al. 2019). Aim of this study is to compare the diet of two *P. intricatus* populations inhabiting alpine rivers characterised by very different environmental quality.

Material and methods

The study was performed in two alpine rivers located in northwestern Italy (Figure 1) and showing very different environmental quality. The first is the river Po at Crissolo (1400 m a.s.l., 44°42'5.95"N, 7°8'52.03"E, hereinafter referred to as Po), an almost pristine running water habitat close to our ALPSTREAM Center. The second is the Dora Riparia at Oulx (1100 m a.s.l., 45° 2'29.54"N, 6°50'5.64"E, hereinafter referred to as Dora), characterised by an evident organic pollution due to sewage discharge. Although they are very similar from a hydro-morphological point of view, the two river reaches greatly differ in water quality (Table 1).

On 17 and 18 April 2018, we sampled with a hand net *P. intricatus* specimens from the two stations early in the morning, because Systellognatha are mainly considered nocturnal feeders (Vaught and Stewart 1974). Moreover, using a Surber net (20 × 20 cm; mesh 255 μm), we collected five samples from each site to assess composition and structure of benthic invertebrate community. All samples were preserved

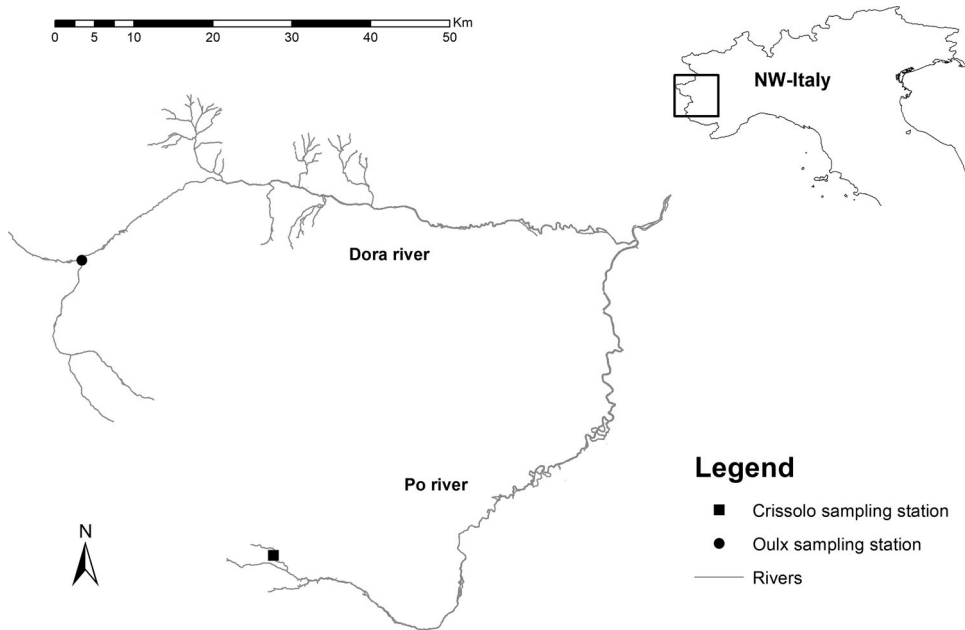


Figure 1. Po and Dora rivers, northwestern Italy. Circle indicates the sample stations.

Table 1. Main abiotic parameters of the two stations.

Parameter	Po	Dora
Conductivity ($\mu\text{S}/\text{cm}$)	95.0	759.0
O_2 (mg/l)	11.1	3.74
Total P (mg/l)	<0.05	0.31
Total NH_4^+ (mg/l)	<0.05	0.92
Mean temperature ($^\circ\text{C}$)	2.89	4.48
pH	7.56	7.89
<i>Escherichia coli</i> (cfu)	2.0	16,418.0

in 95% ethanol, and *P. intricatus* nymphs were measured (total length, femur length, head width, pronotum width) using an ocular micrometer (0.01 mm). For better precision, before measuring, all individuals were pressed flat using a microscope slide. Significant difference in the morphometric measures of *P. intricatus* and richness of benthic taxa in the two sites were statistically tested using the Pearson correlation test and the T-test respectively. In particular, the T-test was performed using the ‘t.test’ R function, which, in case of unequal variance and/or different sample size, is based on the Welch’s t-test that adjusts the number of degrees of freedom. Then, nymphs were processed to assess food consumption by means of gut contents analysis. Guts were removed and the contents of the alimentary canal were analysed by the transparency method for slides (Faure’s fluid) with a NIKON SMZ 1500 light microscope (60–100 X) with JVC TK-C701EG videocamera. Smaller nymphs were analysed without removal of the guts, using the transparency method for slides proposed by Drs. C.L. Bello and M.I. Cabrera (Bello and Cabrera 1999; Tierno de Figueroa et al., 2003; Boggero, Bo, Zaupa, and Fenoglio 2014). Identification of prey was based on sclerotised body parts, particularly head capsules, mouthparts and leg fragments. To

investigate the existence of feeding preferences, we compared gut contents with natural composition and abundance of macroinvertebrate community in the riverbed using the trophic electivity index of Ivlev (1961): $E = (r_i - p_i)/(r_i + p_i)$, where r_i is the relative abundance of a particular taxon in the diet and p_i is the relative abundance of the same taxon in the benthic community. The formula considers the number of taxa (i) found in the diet. The index ranges from -1 to 1 . A value of -1 means total avoidance, 1 indicates preference, and 0 indicates indifference. The occurrence of algae and fine or coarse organic detritus was also recorded. This index was calculated using the function 'ivlev' in the *selectapref* R package (Richardson 2017; R Development Core Team 2017)

Results and discussion

We collected 60 *P. intricatus* nymphs from the Po and 117 from the Dora. Overall, we detected a significant correlation among morphometric measures (Pearson R ranging from 0.793 to 0.827, p always < 0.001). According to this, we decided to use total body length as size indicator, revealing non-significant size difference between the two populations ($t = 1.80$, $df = 72.3$, $p > 0.05$).

Regarding benthic quantitative samplings, we collected 6,691 macroinvertebrates in the Po station, while 8,079 benthic invertebrates were found in the Dora station. On average, the number of benthic taxa was 26 ± 0.77 (mean \pm SE) in the Po station and 10 ± 0.89 (mean \pm SE) in the Dora station. Taxonomic richness in the Po was significantly higher than in the Dora ($t = -13.3$, $df = 4$, $p < 0.001$), while macroinvertebrate density showed an opposite, although not significant, trend. Benthic community in the Po was well structured and diverse, while in the Dora four taxa accounted for 99.2% of the community (Table 2).

During the laboratory analysis, we found 17 guts completely empty in the Dora specimens and 6 in the Po specimens. Analysing gut contents, we noticed that three quarters of the Po nymphs, but only a third of the Dora specimens, showed animal remains. Specimens in the Po sample showed a greater occurrence of coarse particulate organic matter (CPOM) than the ones in Dora, but interestingly the percentage of fine particulate organic matter (FPOM) occurrence was higher in the latter sample (Table 3). Optimal foraging theory postulates that predators select the most advantageous prey according to different factors, such as prey density, energy contents, encounter rate, handling time and similar factors (Krebs 1978). For prey items, we calculated if their presence in the diet was related to an active selection and preference using the Ivlev's index (Figure 2), noticing some interesting elements. First, some organisms, regardless their site-specific density, are always preferred by the two populations of *P. intricatus*. For instance, Chironomidae are positively selected in both stations, independently by their abundance, confirming that these organisms represent a preferred prey for large-sized Systemlognatha, as pointed out by previous studies (Fenoglio, Bo, and Cucco 2005; Fenoglio, Bo, and Malacarne 2007; Fenoglio, Bo, Pessino, and Malacarne 2007; Fenoglio et al. 2009; Quevedo-Ortiz, Fernández-Calero, Luzón-Ortega, López-Rodríguez, and Tierno de Figueroa 2017). By contrast, other organisms that are present in the two environments are never preyed upon,

Table 2. Percentage abundance (% value in the community) of macroinvertebrate taxa collected in the Po and Dora stations (northwestern Italy) during the sampling dates.

Taxa		Po	Dora
Plecoptera			
Leuctridae	<i>Leuctra</i> sp.	31.82	40.99
Nemouridae	<i>Protonemura</i> sp.	3.12	0.03
	<i>Nemoura</i> sp.	0.67	0.04
Taeniopterygidae	<i>Rhabdiopteryx alpina</i> (Kühntreiber, 1934)	2.23	0.00
Chloroperlidae	<i>Siphonoperla montana</i> (Pictet, 1841)	0.16	0.00
Perlodidae	<i>Dictyogenus alpinus</i> (Pictet, 1842)	0.64	0.00
	<i>Isoperla</i> sp.	1.37	0.00
	<i>Perlodes intricatus</i> (Pictet, 1841)	0.06	0.02
Ephemeroptera			
Baetidae	<i>Baetis</i> sp.	5.29	21.17
Heptageniidae	<i>Rhithrogena</i> sp.	9.18	0.00
	<i>Epeorus alpicola</i> (Eaton, 1871)	1.70	0.00
	<i>Ecdyonurus</i> sp.	5.35	0.05
Trichoptera			
Limnephilidae		5.92	0.00
Rhyacophilidae	<i>Rhyacophila</i> sp.	0.07	0.10
Polycentropodidae		0.01	0.00
Hydroptilidae	<i>Agraylea</i> sp.	0.01	0.00
Diptera			
Chironomidae		17.13	28.69
Stratiomyidae		0.03	0.00
Ceratopogonidae		0.09	0.00
Dolichopodidae		0.01	0.00
Muscidae		0.06	0.00
Limoniidae		3.93	0.12
Psychodidae		3.93	0.01
Simuliidae		2.94	0.13
Athericidae	<i>Atherix</i> sp.	0.73	0.00
Empididae		0.09	0.00
Tipulidae	<i>Prionocera</i> sp.	0.13	0.01
Coleoptera		0.00	0.00
Hydraenidae		0.31	0.00
Elmidae		0.13	0.00
Platyhelminthes			
DugesIIDae	<i>Dugesia</i> sp.	0.00	0.02
Planariidae	<i>Crenobia alpina</i> (Dana, 1766)	2.12	0.00
Oligochaeta		0.00	0.00
Lumbriculidae		0.18	0.17
Lumbricidae		0.01	0.00
Naididae		0.13	8.34
Nematoda		0.10	0.11
Aracnidi	Hydracarina	0.28	0.00

Table 3. Percentage of gut of the two *P. intricatus* (Pictet, 1841), populations containing different food items.

Food item occurrence (%)	Po	Dora
Animal	76.7	29.9
FPOM	35.0	73.1
CPOM	10.0	2.2
Algae	3.3	0.7

such as Trichoptera Rhyacophilidae and Diptera Psychodidae. The former perhaps because of their large size and predaceous habits, the latter due to their small size and their habitat preference (i.e., fine organic-rich substrates at the air-water

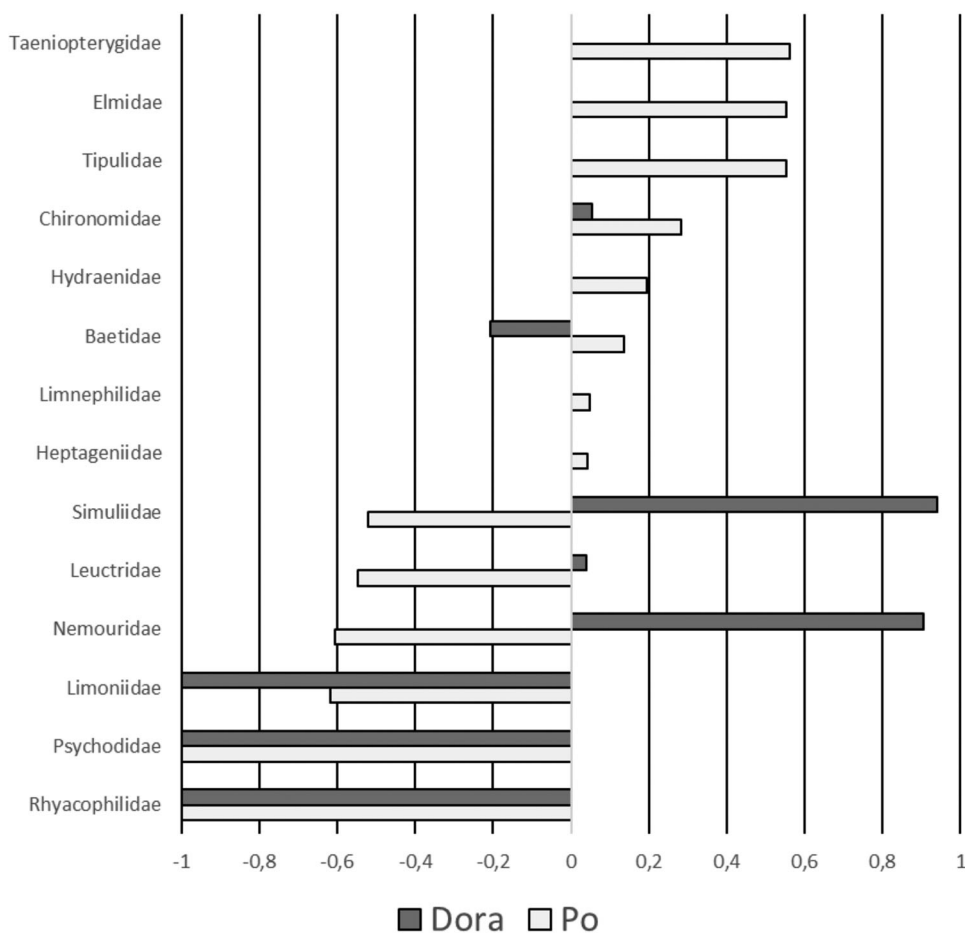


Figure 2. Electivity index (E^*) for macroinvertebrate taxa in the *P. intricatus* (Pictet, 1841) nymphs diet in the two sampling stations.

interface). Numerous taxa that are actively selected as food in the Po (such as Heptageniidae, Limnephilidae, Hydraenidae, Elmidae) do not appear in the diet of the Dora nymphs as they are not present in that benthic community. By contrast, *Perlodes* of the Dora more frequently selected some available taxa, such as Nemouridae and Simuliidae, probably because of the scarcity of other prey. In fact, it is known that under natural conditions, blackflies larvae are uncommon prey for predaceous stoneflies because they are rheostenic Diptera, inhabiting micro-habitats difficult to reach and explore for predators, mainly upper surface of stones in very fast flowing waters. Our study supports the hypothesis that large-sized Systemlognatha nymphs are not strictly carnivorous, but can exhibit some degrees of trophic adaptability. We discovered that *P. intricatus* nymphs can inhabit very different environmental conditions, feeding not only on living animal prey but also on fine and coarse detritus. Interestingly, we evidenced that, while under natural conditions *P. intricatus* nymphs hunt and consume a quite large number of taxa, with some evident selective preference, they also ingest benthic algae (diatoms, see López-Rodríguez, Tierno de

Figuerola, Fenoglio, Bo, and Alba-Tercedor 2009), and small amounts of coarse and fine organic detritus. In a contaminated environment, because of the reduced diversity of the benthic community, *P. intricatus* nymphs show different trophic habits (also if Chironomidae remain the most evidently selected prey). Interestingly, despite their high density in the Dora station, no Naididae were found in the guts: we are quite confident that also if these organisms lack sclerotised structures some traces of them could be identified if positively selected. Probably their small size reduces their profitability for *P. intricatus* nymphs.

In conclusion, we detected that in polluted conditions nymphs: (i) seem not to show differences in size, (ii) include prey items that normally are not selected (probably because of the absence of alternative prey); (iii) ingest a greater proportion of fine particulate organic matter, shifting towards a collector gatherer feeding habit. Considering that no dimensional differences were detected between the two sampled populations, we can conclude that this species can survive and develop in organic polluted environments, due to its trophic plasticity.

Acknowledgements

Authors are very grateful to the anonymous reviewers of their constructive comments. This study was carried out in the context of the 'PITER Terres Monviso Project', supported by the Monviso Natural Park.

Disclosure statement

No potential conflict of interest was reported by the authors.

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